409

X-Ray Characterization of Single Crystals of the Reaction Center I of Water Splitting Photosynthesis

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A new type of crystals of the reaction center I complex, RC I, of water splitting photosynthesis has been prepared from the cyanobacterium Synechococcus sp. In contrast to our first crystals (11), the new ones have a length of up to 1 mm, a thickness of up to 50 μm and show X-ray diffraction patterns with a resolution of about 4 Å. The space group of the hexagonal crystals is probably $P6_322$. One RC I represents the asymmetric unit. The unit cell dimensions are $a = b = 285$ Å, $c = 167$ Å, $\alpha = \beta = 90°$ and $\gamma = 120°$. The building block of the crystal lattice is a trimer (diameter ~19 nm, height ~6 nm, and apparent mass ~600 kDa). The trimer is composed of 3 identical RC I units so that the quaternary structure is α_3. The unit cell harbors four trimers in two layers with two trimers each.

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1. Introduction

A detailed understanding of the function of proteins is only possible together with a knowledge of their structure. Many water soluble proteins have been crystallized and their structure was evaluated by X-ray analysis. Concerning the structure of intrinsic membrane proteins, investigations began only recently because of the difficulties in crystallizing such hydrophobic proteins [1–4]. As yet, only a few such proteins have been crystallized and X-ray analyzed. Regarding the field of photosynthesis, structure analysis down to the 3 Å level was possible for the reaction center of purple bacteria [5, 6]. Special interest is focused also on the reaction centers of plants and cyanobacteria where water is oxidized. In these systems two reaction centers, RC I and RC II, are engaged. They are operating in series between the primary electron donor H_2O and the terminal acceptor $NADP^+$. RC I as well as RC II have been isolated from the cyanobacterium Synechococcus sp. and purified to homogeneity that its outer architecture (size, shape, mass) could be examined [7, 8]. Recently, this was also described for the RC I complex from the cyanobacterium Phormidium laminosum [9]. The RC I complex of both types of cyanobacteria has been crystallized [10, 11]. Our crystals from Synechococcus sp. are dichroic and photo active; clusters of these crystals show X-ray powder diffraction. In this work we describe a new type of large RC I crystal which makes X-ray diffraction of single crystals possible. The patterns obtained render information on the possible space group, unit cell constants and arrangement of the RC I complex within the unit cell.

2. Materials and Methods

Cells of Synechococcus sp. were grown and RC I complexes isolated as described in [12]. The RC I complex was purified through a sucrose-gradient centrifugation [7]. For further purification the RC I complex was solubilized in β-D-maltoside detergent buffer A (MES 0.025 M; $MgCl_2$ 0.02 M; $CaCl_2$ 0.005 M; pH 6.4; 0.03% w/w β-dodecylmaltoside) and applied to a Q-Sepharose (Pharmacia) ion-exchange column at room temperature. The RC I complex was eluted with a $MgSO_4$ gradient [11] and concentrated (a) by precipitation with polyethylene glycol, PEG, resolubilized in buffer or (b) by diafiltration in an "Amicon cell" (XM 300 Membrane).

Crystallization was performed as in [11] at different temperatures (0–50°) with the batch method in Eppendorf tubes or in capillaries. Different concentrations of the precipitating agent PEG 6000 (up to 6% w/w) and salts ($MgSO_4$ up to 0.2 M or NaCl, up to 2 M) were used in the solubilization buffer. The concentration of the complex was characterized through the chlorophyll concentration measured spectroscopically (One RC I is "charged" with about 60 chlorophyll molecules [13]). In contrast to our earlier crystallization procedures where diluted chlorophyll solutions of 25–150 μM were used, the concentrations employed in the present study were up to 100 times higher.

X-ray diffraction patterns from the needle-shaped crystals were taken on the DESY (Hamburg) synchrotron beam line X31 (3.67 GeV, 80 mA) and X11 (5.3 GeV, 30 mA), respectively, using a wavelength of λ = 1.488 Å (Ni-absorption edge) and a collimator size of 0.3 × 0.3 mm. Oscillation photographs were taken on an Arndt-Wonacott camera with flat film cassettes. The crystal-to-film distance was varied from 100 to 150 mm.

3. Results and Discussion

3.1. Crystals

The shape of the crystals obtained differed markedly, depending on salt-, PEG- and protein concentration. Differences in temperature (0–50°C) mainly influenced the speed of crystal growth but practically not the final size and shape. Besides crystals with crude mosaic texture and crystals like rolled sheets, two types of well-shaped crystals have been obtained. As shown in Fig. 1, they are (A) flat hexagonal plates and (B) needles with hexagonal cross section. The needles are mechanically rather stable, grow up to 1 mm in length and 50 μm in diameter. The growth of small crystals (length 40 μm) was observed within minutes. The larger crys-

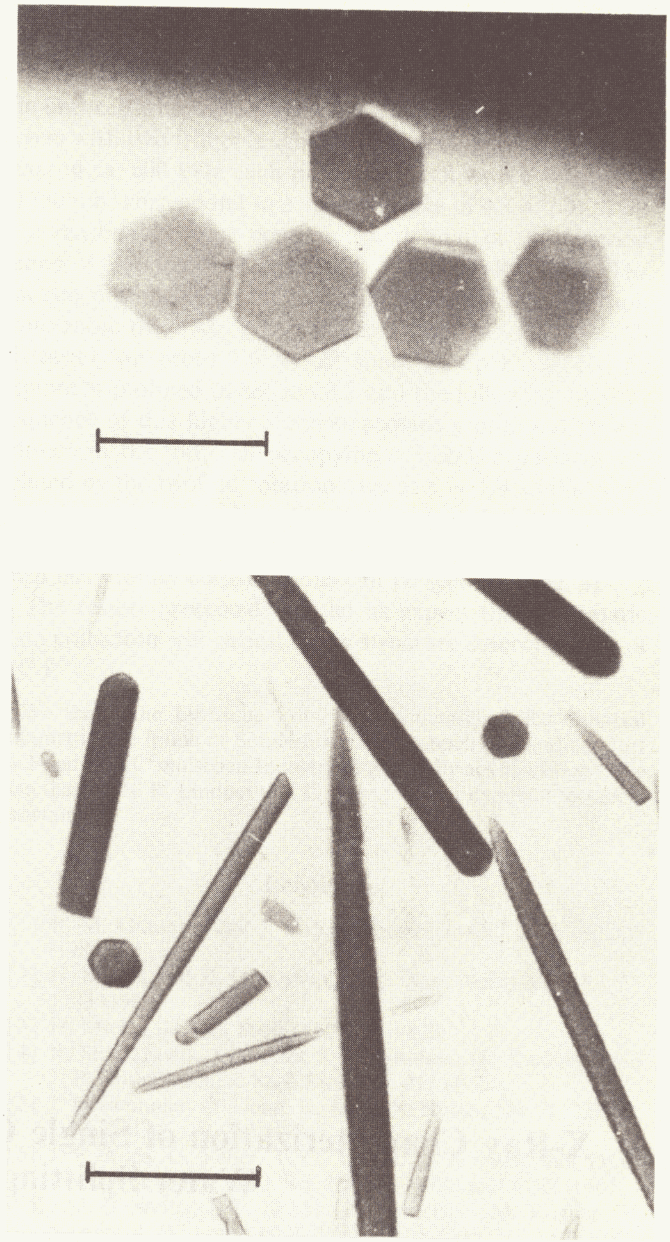

Fig. 1

Top: Hexagonal plates of green RC I crystals grown in a capillary in buffer with 6% PEG 6000 and 2 M NaCl; chlorophyll concentration 0.5 mM. The temperature of 50°C at the beginning was lowered to 4°C within 4 days (the bar indicates 100 μm).

Bottom: Dark green, needle-shaped crystals with hexagonal cross section grown in Eppendorf tubes with low salt- and PEG concentration (range 1–5 mM); chlorophyll concentration 5 mM. 8°C, crystals appeared after 2 days (the bar indicates 100 μm).

tals appear after 1–2 days. Under the polarizing microscope, the crystals appear homogeneous. They display dichroism except in direction perpendicular to the hexagonal face; this indicates a trigonal or hexagonal space group.

3.2. X-Ray Diffraction Analysis

For the X-ray diffraction measurements crystals of the needle-type were used. The diffractions obtained extend to 4 Å resolution (Fig. 2, top). The reflections are sharp and well defined, and the crystals last for several hours in the X-ray beam before the diffraction deteriorates. Several rotation photographs were digitized with a P1000 optronics drum scanner and processed with the Mosco film evaluation system [17] characterising the crystal lattice to be primitive hexagonal with unit cell parameters $a = b = 285(1)$ Å, $c = 167(1)$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$. The crystal c^*-axis coincides with the needle axis. Since the axial reflections $00l$ are extinct when l is odd (Fig. 2, bottom), the possible space groups are $P6_3$ or $P6_322$. In principle, the distinction between these two space groups is made on the basis of $hk0$ photographs. Since the crystals could not be mounted across the capillary, such photographs were not recorded. Instead, 0.5° rotation photographs (not shown) were taken on both sides of the $k0l$ plane, ±(1.75° to 2.25°). They show mirror

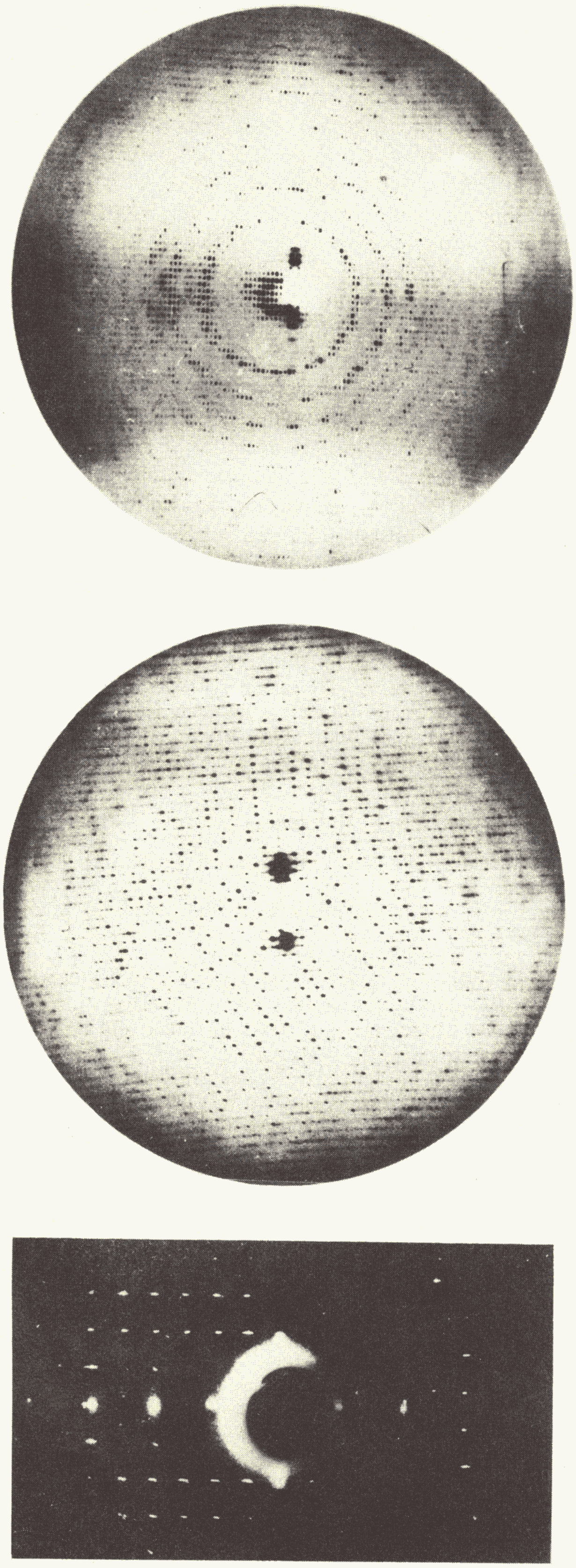

Fig. 2
X-ray diffraction pattern of a crystal showing the overall quality of the diffraction. Top: a^*-axis approx. vertical, c^*-axis horizontal. The resolution extends to 4 Å. Center: crystal in a different position. Bottom: The hhl plane shows clear extinction along the c^*-axis ($00l$, l = odd)

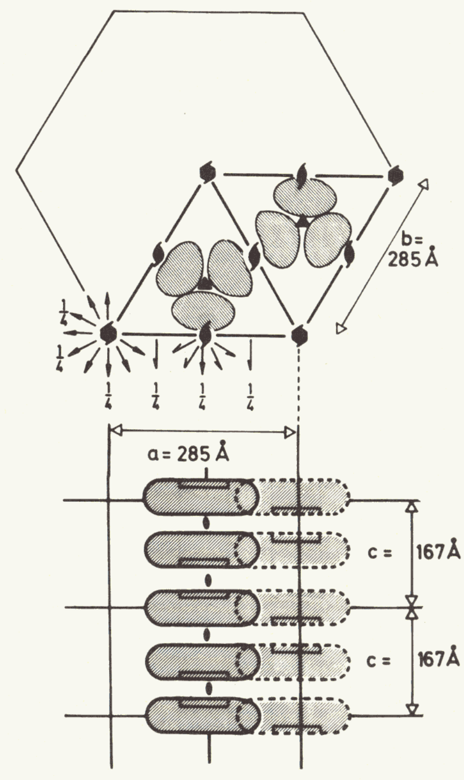

Fig. 3
Possible arrangement of disk-shaped trimer of RC I within the hexagonal unit cell with space group $P6_322$. There is one RC I monomer in the asymmetric unit, its position in the unit cell being determined by the twofold rotation axis at $z = 1/4$.

Top: View on the a, b plane with its symmetry elements and indication of the position of the two RC I trimer complexes.

Bottom: View on the a, c plane and side views of the trimers. The face-to-face arrangement of the trimers are indicated. All symmetry elements except twofold axes at $z = 1/4$ omitted for clarity symmetry which we take as indication for space group $P6_322$. This must be taken with caution as it is based only on some 10 reflections at low glancing angle.

3.3. Building Blocks

The apparent molecular mass of the isolated RC I complex before crystallization has been estimated by gel filtration to be in the range of 600 kDa [7]. Since the position of the gel-filtration profile before crystallization and after re-solubilization of the crystals is exactly the same, the building blocks of the crystal are probably identical with the RC I complex in solution [11]. By aligned and averaged electron microscopic images it was shown that the structure of this isolated complex has a trimeric shape [7, 14]. This has been confirmed recently [9]. The trimer consists of three identical units as shown by biochemical analysis. One unit is identical with one reaction center I [9, 13].

3.4. Architecture of the Crystals

According to the results described above, it is very reasonable to assume that the building block of the crystal is a trimer. According to electron microscopic images, the isolated trimer has a diameter of 19 nm and a thickness of 6 nm, including the detergent belt [7]. Accordingly, the area of two trimers covers 566 nm^2. Since the area of the unit cell is 702 nm^2, the latter represents enough space to incorporate 2 trimers in the a, b plane of the unit cell. They must be arranged such that their local threefold rotation axes coincide with the crystallographic threefold symmetry axes (Fig. 3, top). This implies that the three monomers in the trimer are identical so that the quaternary structure is α_3.

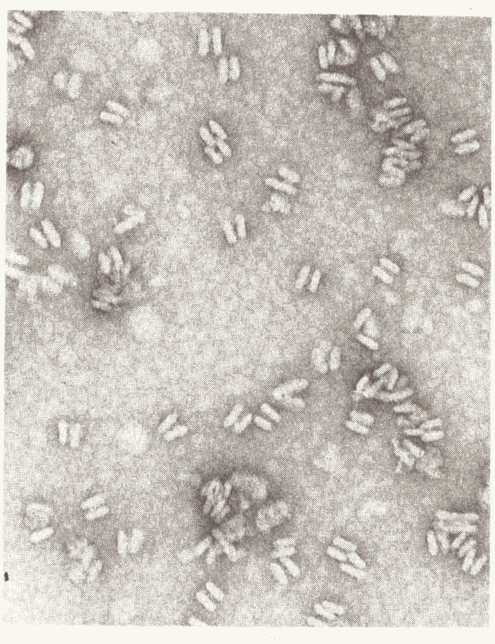

Fig. 4
Electron micrograph of sideview projections of trimers of RC I in solution negatively stained with 1% uranyl acetate [16]. Preferential face-to-face arrangement of two trimers in form of sandwiches is evident

Since the thickness of the RC I trimer disk is ca. 6 nm, the unit cell with a c-axis length of 16.7 nm can harbor two layers with two trimers each. Four trimers with an apparent mass of ca. 600 kDa each in one unit cell with a volume of 11750 nm^3 correspond to a specific space of 4.9 $Å^3$/Da. This is a reasonable value compared with that of the reaction center of pseudomonas viridis (5.8 $Å^3$/Da, see Ref. [15]). The packing arguments for the unit cell do not provide a decision concerning the space group of the crystals, $P6_3$ and $P6_322$. However, we prefer the $P6_322$ space group based on the argument outlined in section 3.2 and the following: A consequence of this higher symmetry space group is that two trimers in the unit cell occupying 1/3, 2/3, z position are related by the twofold rotation axes at $z = 1/4$ so that they are arranged face-to-face (Fig. 3, bottom). This is in agreement with electron microscopic side views of trimers where such face-to-face constellations can be seen (see Fig. 4).

The results presented here let us expect that systematic data collection will permit X-ray structure determination of RC I.

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